APPENDIX A

CURRICULUM VITAE

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EDUCATION

1967 BA	Hunter College, New York, NY (New York State Regents Scholarship)
1968-1969	University of Barcelona
1969-1976	Free University of Brussels, Belgium
1972	Candidature in Medical Sciences
1976	Cum Laude Doctorate in Medicine

POSTDOCTORAL TRAINING

Internship and Residencies

1976-1977	Internship: Beth Israel Medical Center, (BIMC), New York, NY
1977-1978	Junior Residency: BIMC, New York, NY
1978-1979	Senior Residency, Assistant Chief Resident: BIMC, New York, NY
1979	Resident in Research and Assistant Physician to the Rockefeller University Hospital, New York, NY (4/79-6/79)

Research Fellowships

1979-1980	Clinical Fellowship in Hematology Oncology, Beth Israel Hospital, Boston, MA
1980-1982	Research Fellowship in Hematology Oncology, Beth Israel Hospital and Harvard Medical School, Boston, MA

Licensure and Certifications

New York State #132383
Massachusetts #44896
California #C 042482
Pennsylvania #MD-045661 -L

Boards

ABIM #70582
Hematology and Oncology, B/E

Society Memberships

ASH
ASCO
DIA

Society of Biological Therapy
ABPI - Biotechnology Committee, 1990-1991
PMA - Biologics Section/AIDS Fellow, The Massachusetts Medical Society
AACR
American Association for the Advancement of Science

ACADEMIC AND PROFESSIONAL APPOINTMENTS

1997-Present	Head, Medical Affairs Cell Therapeutics, Inc. Seattle, WA
1992-1997	Vice President, Clinical Development Chiron Technologies Chiron Corporation Emeryville, CA
1994-Present	Assistant Clinical Professor Department of Medical Oncology UCSF Cancer Center, Mt. Zion San Francisco, CA
1992	Attending Physician Fox Chase Cancer Center Philadelphia, PA
1991-1992	Director Clinical Research Oncology Sterling Winthrop Pharmaceutical R&D Malvern, PA
1990-1991	Director Sterling Oncology, Europe Guildford, UK
1989-1990	Senior Director, Acting Head Clinical Research and Development CETUS Corporation Emeryville, CA
1986-1989	Associate Director Clinical Research and Development CETUS Corporation Emeryville, CA
1982-1986	Staff Addison Gilbert Hospital, Gloucester, MA Beth Israel Hospital, Boston, MA

1984-1986	Field Liaison Physician Commission on Cancer American College of Surgeons
1984-1986	Chairman, Oncology Committee Addison Gilbert Hospital Gloucester, MA
1982-1986	Medical Director Hospice Visiting Nurse Association of the North Shore Gloucester and Beverly, MA
1982-1986	Private Practice Rockport, MA
1981-1982	Instructor in Medicine Harvard Medical School Boston, MA
1978-1979	President of the House Staff Association Beth Israel Medical Center New York, NY
1977-1979	Member Medical Board Beth Israel Medical Center New York, NY

GRANTS AND AWARDS

Grant number FDR-000358-02 awarded September 1988, by the FDA Orphan Products Division for Clinical Research in Primary T-Cell Immunodeficiencies.

COURSES TAUGHT

1994-1996	University of California - Berkeley (Extension) Drug Development

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Publications available upon request.

MARGOLIN

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APPENDIX B

REF# 123

Prospective Randomized Trial of Lisofylline for the Prevention of Toxicities of High-Dose Interleukin 2 Therapy in Advanced Renal Cancer and Malignant Melanoma

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ABSTRACT

The therapeutic application of high-dose interleukin (IL) 2 in human malignancy is limited by severe multiorgan toxicities that are mediated, in part, by tumor necrosis factor (TNF) and IL-1. CT1501R (lisofylline; LSF) is one of several methyl xanthine congeners that inhibit the effects of TNF by the interruption of specific signal transduction pathways. This randomized, placebo-controlled trial was designed to assess the activity of LSF in reducing the toxicities of high-dose IL-2 therapy.

Fifty-three patients with metastatic renal cancer or malignant melanoma were treated with i.v. bolus IL-2, 600,000 IU/kg every 8 h for 5 days (14 doses), followed by 9 days of rest and another 5-day course of IL-2. Patients were randomly assigned to LSF, 1.5 mg/kg i.v. bolus, or placebo every 6 h during IL-2 therapy. All patients were to be treated to individual maximum tolerance of IL-2 at the intensive care unit level of support. The end points for statistical analysis were the number of IL-2 doses administered during the first cycle of treatment (maximum, 28) and the toxicities experienced by each group after the first 8 planned IL-2 doses.

There was no difference between the LSF and placebo groups in the mean number of IL-2 doses tolerated in the entire first cycle of therapy (19.6 ± 5.4 *versus* 19.5 ± 5.8, $P = 0.86$) or in the first or second 5-day course of IL-2. The only significant difference in toxicities occurring through the eighth dose of IL-2 was in the maximum elevation of serum creatinine (mean, 1.7 ± 0.8 for placebo *versus* 1.5 ± 0.6 mg/dl for LSF, $P = 0.013$). A Monte Carlo analysis of major toxicities over the first 14-dose course of therapy showed a statistically significant difference favoring the LSF-treated group ($P = 0.025$). LSF was well tolerated, associated only with mildly increased nausea ($P = 0.006$ after eight IL-2 doses, but not significant for the entire first cycle). The antitumor activity was comparable in both groups (objective responses, 2 of 28 with LSF *versus* 4 of 24 with placebo). The mean peak plasma concentrations of LSF on days 1, 5, and 19 were 6.24, 3.83, and 5.04 μmol/liter, respectively.

In conclusion, with this dose and schedule, LSF did not alter the toxicities of high-dose i.v. IL-2 sufficiently to impact the overall dose intensity of IL-2. Successful IL-2 toxicity modulation may require the use of higher doses of LSF, the development of agents with more potent anti-TNF activity, and/or combined modulating agents that function via distinct mechanisms to interrupt cytokine-mediated signaling.

INTRODUCTION

IL-2[2] is the most active systemic therapy for advanced renal cancer. It also has activity against melanoma and some hematological malignancies. Based on the dose dependency of IL-2 in animal models, most clinical trials of IL-2 have administered high doses that can only be tolerated by cancer patients with excellent performance status and organ function.

Patient tolerance of high-dose IL-2 is limited by the development of multiorgan toxicities that are dose and duration related (1). The constellation of toxicities associated with high-dose IL-2 therapy includes a generalized "vascular leak syndrome," which is believed to be mediated by nitric oxide produced in response to the local effects of cytokines such as TNF, IL-1, and IFN-γ (2–4). The safe use of this agent requires extensive experience in the use of biological agents and the management of the multiorgan toxicities associated with the vascular leak syndrome (1, 5).

This study was designed to test the possibility that a suppressor of the acute inflammatory response in patients undergoing therapy with high-dose IL-2 would improve the toxicity

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[2] The abbreviations used are: IL, interleukin; TNF, tumor necrosis factor; LSF, lisofylline; PTX, pentoxifylline; CTI, Cell Therapeutics, Inc.; LAK, lymphokine-activated killer; ARDS, adult respiratory distress syndrome; TNFR:Fc, TNF receptor:IgG chimera; BRMP, Biological Response Modifiers Program of the National Cancer Institute.

profile of this therapy. LSF is a chiral secondary alcohol in the *R* configuration and is not a normal metabolite of the xanthine ester, PTX. Unlike PTX and its normal metabolic product, M1, which is the *S* enantiomer of the secondary alcohol, LSF inhibits a specific signal pathway activated by TNF-α and other pro-inflammatory cytokines through suppression of formation of specific species of phosphatidic acid and secondary inhibition of activation of stress-activated protein kinase (6). LSF does not inhibit the activation of MAP kinase or stress-activated protein kinase 2 and affects a pathway that is different from the arachidonate-dependent species involved in the polyphosphoinositide-directed second messenger pathways. Several inflammatory cytokines (TNF, IL-1, and platelet-activating factor) signal through this pathway, which is also activated in response to cell-damaging stimuli, such as radiation, heat, endotoxin, or cytotoxic agents, and quiescent under normal homeostatic conditions (6).

In the earliest clinical trials, PTX was evaluated for its ability to modulate the toxicities of bone marrow transplantation, which have been correlated with plasma TNF-α levels (7). The plasma levels of M1, as measured in a nonchiral assay, achieved during PTX therapy correlated with the apparent reduction in transplant regimen-related toxicities compared with matched historical control patients, but PTX administration at the doses required to achieve target plasma levels of M1 caused unacceptable nausea and emesis (8). In a recent dose-escalation study of PTX in patients receiving IL-2 by continuous i.v. infusion, ciprofloxacin was coadministered in an effort to modulate PTX metabolism (9). Although up to 10% of the M1 was LSF, its formation was rate limited, because raising the dose of PTX to toxic levels failed to increase plasma LSF concentrations. LSF was then tested in Phase I trials seeking to establish a therapeutic dose level based on target plasma levels and clinical side effects.

Phase I trials of LSF in various combinations with chemotherapeutic or biological agents were based in part on the achievement of target plasma levels in normal volunteers in the range shown *in vitro* and in animal models to modulate inflammatory end points (10). The dose-limiting toxicities of LSF are nausea, headaches, and flushing at the maximum tolerated doses of 5 mg/kg by 10-min i.v. infusion every 6 h (Refs. 11–13 and data on file at CTI). We selected a dose, 1.5 mg/kg every 6 h, that was projected to yield peak plasma levels in the range of 6–9 μmol/liter. Our study was designed to compare specific organ toxicities between LSF-treated and placebo groups. The usual dose-limiting toxicities of IL-2 are hypotension, renal insufficiency, fluid retention with respiratory compromise, central nervous system dysfunction, and cardiac events such as arrhythmias or myocarditis (1, 14, 15). Because high-dose IL-2 regimens generally provide for a larger number of planned doses than can be tolerated by most patients, the most important indicator of IL-2 tolerance is the number of total IL-2 doses, or percentage of planned doses, that can be safely administered. The effect of a toxicity modulator can be measured by the ability to deliver more IL-2 at an expected level of toxicity or to reduce the toxicities of a fixed amount of IL-2.

PATIENTS AND METHODS

Patient Selection. Patients were required to have a diagnosis of renal cell carcinoma or malignant melanoma with measurable or evaluable metastatic disease. Additional eligibility requirements included Eastern Cooperative Oncology Group performance status 0 or 1, age ≥18 years, WBC count ≥3,500/μl, platelet count ≥100,000/μl, hemoglobin ≥10 gm/dl, serum creatinine ≤1.5 mg/dl (or clearance ≥60 ml/min), serum bilirubin ≤1.5 mg/dl, serum transaminases ≤5 times the upper limit of normal, and adequate pulmonary function (FEV_1 ≥2.0 liters or 75% of predicted). Patients were excluded if they had prior exposure to IL-2, history of cardiovascular disease (cardiac stress test required for all patients over 40 or with medical risk factors), central nervous system metastasis or seizure disorder, or serological positivity for hepatitis B surface antigen, human immunodeficiency antibody, or pregnancy. Patients with a history of methylxanthine intolerance or hypersensitivity were also excluded. All patients provided voluntary written informed consent, and the protocol and consent form were approved by the institutional review board at each of the participating centers.

Drug Supply and Preparation. Recombinant IL-2 (Chiron Corporation, Emeryville, CA) was provided as a 5-ml vial containing 1 mg protein, with a specific activity of 18 million IU/mg. The contents of each vial was reconstituted with 5 ml of sterile water, and the final dose was diluted into 50 ml of dextrose (5%) in water and administered as an i.v. infusion over 15 min.

LSF (IND 341928; CTI) was provided as a sterile solution of LSF in water at a concentration of 60 mg/ml in 20-ml glass ampules. The calculated dose of drug or placebo was mixed with 50 ml sterile 0.9% sodium chloride and administered i.v. over 10 min. A 5-ml normal saline flush was given before and after each dose of LSF or placebo. Blinded randomization was performed by the investigational drug pharmacist at each institution at the time of patient enrollment into the study, and neither the patient nor the physician was informed of the drug assignment.

Treatment Plan. IL-2 was administered in the clinical research center or intensive care unit of the participating hospitals, all of which had extensive prior experience with high-dose IL-2. A cycle of therapy consisted of IL-2, 600,000 IU/kg every 8 h for a maximum of 14 doses over 5 days (days 1–5), followed by 9 days of rest (days 6–14), and a second course of 14 doses of IL-2 over 5 days (days 15–19). LSF (1.5 mg/kg) or placebo was given every 6 h, starting 8 h before the administration of the first dose of IL-2 (so that two doses of study drug preceded the first dose of IL-2) and continuing until one dose following the last dose of IL-2 on each 5-day course.

Patients were required to take acetaminophen (650 mg p.o. every 4 h during the administration of IL-2), indomethacin (25 mg p.o. prior to and every 6 h during the administration of IL-2), and ranitidine (150 mg p.o. or 50 mg i.v.) every 12 h. Medications available for symptom management included anti-diarrheal agents, anti-emetics (excluding glucocorticoids), antihistamines for pruritus, and meperidine for chills. The use of supplemental albumin infusions, HCO_3 solutions for the treatment of acidosis, and dopamine or phenylephrine for the management of hypotension were at the discretion of the treating physician. All of the patients with central venous catheters were

required to receive broad-spectrum antibiotic coverage reflecting the institutional patterns of nosocomial infection and antibiotic sensitivities, but ciprofloxacin was not permitted because its potential interaction with LSF (10).

Dose Adjustments for Toxicity. All doses of IL-2 were administered at the original dose level, and full doses were withheld when indicated for the management of toxicities, with resumption of IL-2 therapy upon adequate resolution of toxicity. Omitted doses were not replaced, and a course of therapy was never extended beyond the planned 14 doses over 5 days. The following guidelines for the management of toxicity were identical to those published previously by our group (1).

Treatment with IL-2 was to be permanently discontinued in the event of sustained ventricular tachycardia or other dysrhythmia unresponsive to medical therapy; EKG changes or chest pain consistent with ischemia or enzyme elevations suggestive of ischemia or myocarditis; respiratory failure requiring intubation; renal dysfunction requiring dialysis; coma or toxic psychosis lasting more than 48 h; or major gastrointestinal complication (hemorrhage, perforation, or ischemia).

One or more doses of IL-2 were to be withheld for toxicities less severe than those listed above and could be resumed upon adequate toxicity resolution. These included supraventricular tachyarrhythmias responsive to medical management; hypotension worsening despite vasopressor therapy; respiratory dysfunction (severe dyspnea or hypoxemia); mild to moderate mental status changes; renal dysfunction with oligoanuria and/or metabolic acidosis or other electrolyte disturbances; or severe skin rash with moist desquamation or bulla formation. The decisions to withhold and resume IL-2 were based on the patient's overall status, timing and severity of the toxicity, and degree and rapidity of resolution upon withholding one or more doses of therapy.

The maximum number of LSF study drug doses per course was 20, and this drug was not withheld for temporary IL-2 withholding until all IL-2 dosing was completed for that cycle. There were no planned dose adjustments for the LSF study drug, but any toxicity occurring during therapy and strongly suspected to be related to this agent rather than IL-2 or concomitant medications was to be managed by withholding one or more doses of LSF/placebo.

Laboratory and Clinical Assessments. The complete blood count and chemistry tests were repeated daily during active treatment and until the resolution of laboratory abnormalities following each treatment cycle. These tests were also performed at weeks 7 and 11 of each cycle.

Tumor measurements for the assessment of response were performed at approximately weeks 7 and 11, and patients with evidence of antitumor activity were eligible to receive one or at most two additional cycles of IL-2 with the same study drug to which they had originally been randomized, at approximately 12-week intervals. The definitions of response have been described previously (14).

Plasma for LSF determination was drawn on days 1, 5, and 19 before and immediately following completion of the LSF infusion. Plasma for cytokine assays (TNF-α and IFN-γ) was drawn on day 1 before treatment and on days 5 and 19 at 2 h following the first morning dose of IL-2. In the case of patients who had to discontinue IL-2 therapy before receiving all of the planned doses, the final sample was drawn on the morning of the last day the patient was expected to receive IL-2 after continuous or near-continuous dosing. TNF-α and IFN-γ concentrations were measured using ELISA kits from Endogen (Boston, MA) or Genzyme (Cambridge, MA). The manufacturer's instructions were followed for each kit.

Serum levels of free fatty acids and the ratio of the C18 unsaturated fatty acids linoleate and oleate to the C16 fully saturated palmitate were assayed using the methods of Bursten *et al.* (6) before treatment and on day 5.

Plasma concentrations of LSF and the metabolites CTI501S and CT1537 were determined by a validated, sensitive chiral high-performance liquid chromatographic assay with UV detection. The lower limit of quantitation of the assay is 5 ng/ml (18 nmol/liter).[3]

Study Design and Statistical Plan. The objective of this study was to assess the ability of LSF to reduce the toxicities associated with high-dose bolus i.v. IL-2. Toxicity was analyzed by system and grade, using the BRMP toxicity grading scale. The comparison of total IL-2 administered on the first cycle of therapy was based on the intent to treat all patients to individual maximum tolerated IL-2. In prior studies, we had demonstrated that the average number of IL-2 doses tolerated on a two-course cycle of therapy was 20 of 28 planned, with only 10–15% of patients able to tolerate all 28 doses (5, 14). To directly compare the effect of LSF on the specific organ toxicities of IL-2, we also performed a comparison of selected toxicities at a time point at which all patients were likely to have received all of the planned doses (after eight doses of IL-2 were administered or were due to be administered). In this prospective statistical plan, a sample size of 50 patients was selected to detect a four-dose difference in the mean number (estimating a SD of 3–4) of IL-2 doses tolerated during the first treatment cycle with 95% significance and 80% power. This patient number would also allow us to detect an increase from 15 to 40% in the percentage of patients able to tolerate all of the 28 planned IL-2 doses on the first full cycle of therapy.

After the completion of this study, we re-analyzed the data using the Monte Carlo analysis of "primary efficacy parameters" (16) to detect a possible difference between the treatment groups when multiple organ toxicities were compared during the first course of IL-2 therapy. This plan was developed after the study was completed but before the data were unblinded. The statistical methods used for this analysis are provided in the "Appendix."

RESULTS

Patient Characteristics. Between March and November, 1994, 53 patients were registered into this trial, 32 with metastatic renal carcinoma and 21 with malignant melanoma. After pre-stratification for histology and, in the renal cancer group, nephrectomy status, 29 patients were randomly assigned to receive LSF and 24 to placebo. After randomization, one patient with renal cancer who had been randomized to receive

[3] CTI, unpublished data.

Table 1 Demographic data

	LSF	Placebo
No. of patients	28	24
Renal carcinoma	16	15
Melanoma	12	9
Age (median, range)	47 (24–70)	51 (30–67)
Median performance status	0	0
PS = 0	22	17
PS = 1	6	7
Prior therapy		
Renal		
Nephrectomy	13	13
Interferon	5	1
Chemotherapy	1	
Melanoma		
Chemotherapy	3	1
Biological response modifier (Vaccine, BCG, Cytokine)		
Biological response modifier alone	2	0
In combination with chemotherapy or in sequential regimens	1	7

Table 2 IL-2 doses by course: LSF *versus* placebo

	LSF	Placebo	*P*
No. of IL-2 doses administered (median, range)			
By the 8th scheduled dose	8.0 (4–8)	7.0 (4–8)	0.022
Course 1	12 (5–14)	12 (5–14)	0.773
Course 2	8.5 (4–14)	9.5 (3–14)	0.756
Entire cycle	20 (5–28)	20 (5–28)	0.861
Cumulative IL-2 administration per kg baseline weight (median, range)			
By 8th scheduled dose	4.8 (2.4–4.9)	4.2 (2.4–4.9)	0.002
Course 1	7.2 (3.1–8.6)	7.0 (3.1–8.4)	0.36
Course 2	5.1 (2.4–8.4)	5.4 (1.7–8.4)	0.90
Entire cycle	12.0 (3.1–16.8)	11.6 (3.0–16.8)	0.62

LSF was found to be ineligible based on preexisting ventricular arrhythmias and removed from the study. The remaining 52 patients, all of whom were eligible and evaluable for toxicity and response to therapy, form the basis of this report. The demographic and prior therapy data are provided in Table 1. Patients were not heavily pretreated; only 7 of 31 renal cancer patients had received prior systemic therapy, and 12 (57%) of the melanoma patients had received standard non-IL-2-containing chemotherapy or biochemotherapy combinations.

Toxicity Analysis. The comparisons of administered IL-2 and LSF study drug for the two cohorts are shown in Table 2. Toxicities were compared after the first eight scheduled doses. Despite the selection of this time point for full IL-2 administration in both groups, there was a statistically significant difference in the median number of IL-2 doses by this time point favoring the LSF treatment group (8.0 *versus* 7.0, $P = 0.023$); however, this advantage was no longer evident by the end of the first full 5-day cycle of scheduled dosing. There was no significant difference in the amount of IL-2 received by the two groups when analyzed by course or for the first full treatment cycle. There was also no difference at any of these time points in the number of LSF or placebo doses administered to the two patient groups when analyzed at any time point. Two patients in each group were able to receive all 28 planned doses of IL-2 in the first cycle.

Table 3 Comparison of selected toxicities after the 8th scheduled dose

	LSF	Placebo	*P*
Peak serum creatinine	1.5 ± 0.6	1.7 ± 0.8	0.013
Maximum rise in serum creatinine *vs.* patient baseline	0.4 ± 0.4	0.5 ± 0.8	0.331
Hypotension			
Grade 2	6	8	0.076
Grade 3	8	11	
Grade 4	0	0	
Cardiac toxicity			
Grade 2	0	4	0.389
Grade 3	0	0	
Grade 4	2	1	
Neurotoxicity grade			
Grade 2	5	2	0.156
Grade 3	0	4	
Grade 4	0	0	
Nausea/Emesis			
Grade 2	15	10	0.006
Grade 3	5	0	
Grade 4	0	0	
Bilirubin			
Grade 2	4	5	0.424
Grade 3	0	0	
Grade 4	0	0	

Toxicity analysis focused on those toxicities that have been dose limiting in prior studies of high-dose IL-2. Table 3 shows the comparative frequencies of hemodynamic, renal, neurological, cardiac, pulmonary, and gastrointestinal toxicities by grade, observed through dose eight of IL-2.

Among these selected parameters, there were few differences that reached statistical significance between the LSF and placebo groups. The peak serum creatinine was significantly higher in the placebo group (mean, 1.7 ± 0.8 for placebo *versus* 1.5 ± 0.6 mg/dl for LSF, $P = .013$), but the changes from baseline serum creatinine did not differ significantly between treatment groups.

The occurrence of nausea and vomiting was significantly greater in the LSF group only at the eighth-dose analysis ($P = 0.006$) and did not show a significant difference at any of the other time points; although nausea is a known side effect of the methyl xanthines, it also occurs frequently and in a cumulative fashion with IL-2, an effect that may have masked the detection of a difference between LSF and placebo at later time points.

The results of the analysis of primary efficacy parameters are shown in Table 4. The average total score is a value derived from the maximum toxicities of the systems selected for analysis and the duration of maximum toxicity. To determine this, the maximum toxicity grade on each of the first 5 days of treatment for each organ system was summed to generate an overall score for each system. With the exception of maximum change from baseline serum creatinine, the other values are maximum toxicity grades for each individual system. These results demonstrate that when analyzed as a linked group over

Table 4 Primary efficacy parameters analysis, course 1

		LSF $N = 28$	placebo $N = 24$	P
Average total score	Mean[a]	1.1 (1.3)	1.9 (1.6)	0.053
	Median[b]	1 (0–5)	2 (0–5)	
Maximum change from baseline in serum creatinine	Mean	1.0 (1.1)	1.0 (0.9)	0.338
	Median	1 (0–5)	1 (0–4)	
Maximum creatinine toxicity	Mean	0.9 (0.8)	1.1 (0.8)	0.144
	Median	1 (0–3)	1 (0–3)	
Maximum hypotension toxicity	Mean	1.8 (1–2)	2.4 (0.9)	0.020
	Median	2 (0–3)	3 (0–3)	
Maximum cardiac toxicity	Mean	0.9 (1–2)	1.0 (1.1)	0.362
	Median	1 (0–4)	1 (0–4)	
Maximum neurological toxicity	Mean	1.4 (0.9)	1.5 (1.0)	0.398
	Median	1 (0–3)	1 (0–3)	
Maximum pulmonary toxicity	Mean	1.3 (1.3)	1.4 (2.4)	0.481
	Median	1 (0–4)	1 (0–4)	
Minimum P				0.020
Randomization distribution P				0.036

[a] Numbers in parentheses are SD.
[b] Numbers in parentheses are the ranges.

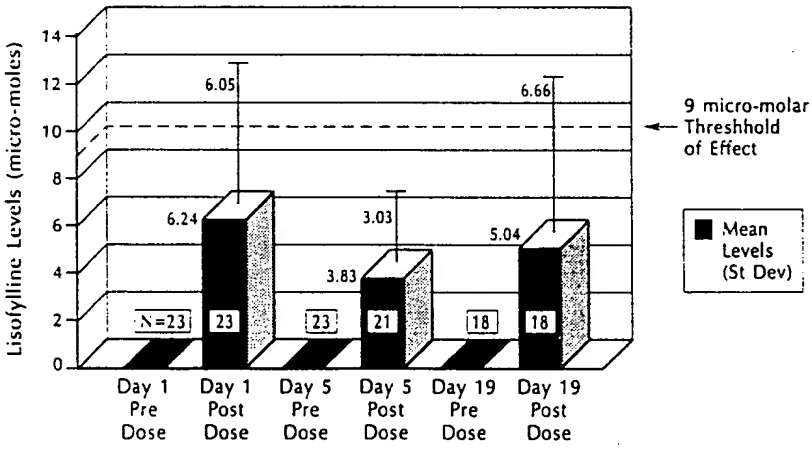

Fig. 1 LSF levels pre- and postdose on days 1, 5, and 19. LSF determinations were performed as described in "Patients and Methods."

the entire first treatment course, the overall organ system toxicities were less severe in the LSF group than in the placebo group ($P = 0.053$ for average total score and $P = 0.036$ for randomization distribution).

Antitumor Responses. All patients were assessed for objective response at week 11 (8 weeks following the completion of the first cycle of therapy). There was one complete (melanoma) and one partial (renal) response among the 28 patients assigned to LSF and one complete (melanoma) and three partial (renal) responses among the 24 assigned to placebo. This difference was not statistically significant ($P = 0.61$).

Pharmacological Analyses. LSF levels before therapy and peak posttherapy levels from days 1, 5, and 19 are shown in Fig. 1, which demonstrates that the median plasma levels were below the targeted therapeutic range of 9 μM.

Plasma levels of γ-IFN levels were measured on day 5 of course 1 in 26 patients (7 placebo and 19 LSF). The median levels for placebo and LSF patients were 211 (0–474) and 119 pg/ml (0–282), respectively ($P = 0.069$). TNF levels, measured in two separate analytic laboratories, were undetectable.

Serum free fatty acid analysis was performed in 24 patients (12 each on placebo and LSF) on days 1 and 5 of the first treatment course. Patients receiving placebo had a day 1 average acyl ratio of 1.14 ± 0.41, rising to 1.53 ± 0.32 by day 5 ($P < 0.05$, Mann Whitney). LSF patients had a day 1 average acyl ratio of 1.27 ± 0.3 ($P > 0.10$ *versus* day 1 placebo patients), which fell to 1.21 ± 0.21 ($P > 0.10$ *versus* day 1 LSF; $P < 0.02$ *versus* day 5 placebo). The results of the day 1–day 5 changes in acyl chain ratios are illustrated in Fig. 2. Fig. 3 shows the results for the three major serum acyl chains linoleate, oleate, and palmitate on day 5. Patients receiving placebo demonstrated increases of >600% in each of these determinations, whereas this increase was significantly reduced ($P < 0.001$, Mann-Whitney) to 140–150%, but not abrogated, in patients randomized to receive LSF.

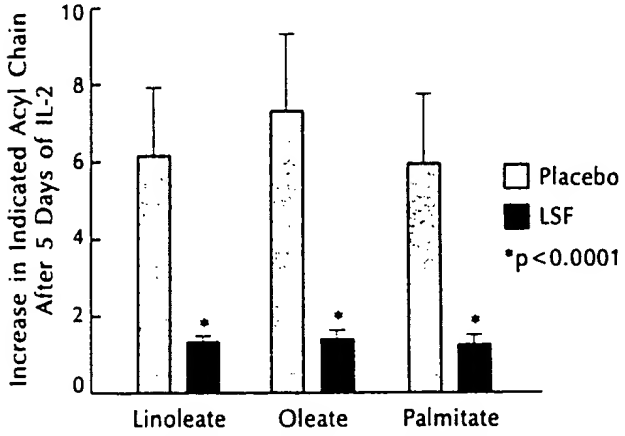

Fig. 2 Lipid extraction and analysis were performed as described previously (24).

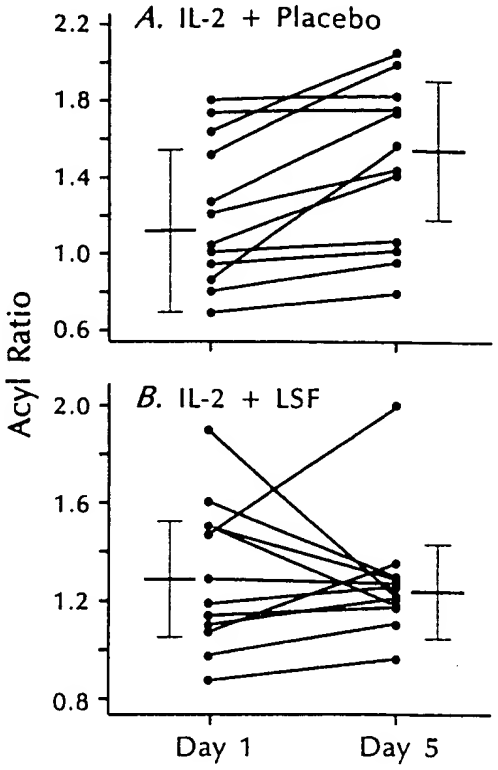

Fig. 3 *A*, acyl ratio values in patients receiving IL-2 plus placebo. *B*, acyl ratio values in patients receiving IL-2 plus LSF. *Bars*, mean ± SD.

DISCUSSION

In this randomized, placebo-controlled trial, we observed minimal beneficial effects of LSF administration on the parameters selected to represent the toxicity end points of high-dose IL-2 therapy. Specifically, LSF did not permit the administration of more IL-2 and did not substantially alter the toxicity profile of a fixed dose of IL-2. The comparison of toxicities attributable to LSF confirms that the active treatment cohort received virtually all of the planned LSF study drug. Thus, the lack of appreciable toxicity reduction by LSF was not due to LSF toxicity, leading to a reduction in the IL-2 doses tolerated in this treatment cohort.

Preclinical data suggesting a role for the methyl xanthines in high-dose cytokine-based therapies for cancer provided the biological rationale for the recent clinical trials. Edwards *et al.* (17) demonstrated that when PTX was used in the C57Bl/6 murine model of IL-2-induced regression of pulmonary metastases from the MCA-105 sarcoma, the antitumor efficacy of IL-2 was preserved while treatment toxicities were reduced. This included reduction in the severity of IL-2-induced multiorgan edema, hepatic dysfunction, leukopenia, and thrombocytopenia (17).

These findings provided support for the design of protocols to test the ability of this agent to reduce the multisystem toxicities of high-dose IL-2 therapy in patients with cancer while avoiding the loss of antitumor activity. Prior to the availability of LSF, Thompson *et al.* (18) performed a Phase Ib trial of PTX and IL-2 with the addition of ciprofloxacin to maximize the levels of M1R. The study design consisted of a dose escalation of i.v. PTX to further investigate the dose-limiting toxicities and correlate plasma levels of PTX and its M1 and M1R metabolites with the tolerance and toxicities of IL-2 and LAK cells in patients with metastatic renal cancer (18). The dose-limiting toxicities in this study were nausea and atrial arrhythmias, and there was a significant correlation between plasma levels of PTX and the quantity of IL-2 tolerated. There was a lower incidence of azotemia and acidosis compared with historical controls. There was no difference in the lymphocyte rebound following completion of the high-dose IL-2 infusion and no difference in LAK cell numbers or cytolytic activity; the changes in plasma TNF-α levels were variable and could not be compared with those of the small number of historical controls, but the authors recognized that even the highest plasma PTX levels achieved in this study were far lower than those required for inhibition of TNF-α production (19, 20). LAK cell precursor activity (ability to stimulate *in vitro* the peripheral blood lymphocytes of patients treated with IL-2 to cytolytic activity against natural killer cell-resistant tumor cells) was not suppressed by PTX treatment *in vivo* or by *in vitro* exposure (21). The objective response rate of 17% (three objective responses among the 18 patients) was comparable to the historical response rates of 15–25% for this form of therapy.

Anderson *et al.* (22) began a trial of toxicity modulation of high-dose infusional IL-2 (18 mIU/m^2/day during 4 days/week for 4 weeks) with PTX, 400 mg five times daily. After only five patients were treated, the trial was closed because of the lack of favorable effect on IL-2 toxicity and the marked increase in gastrointestinal toxicity due to PTX (22).

Thompson *et al.* (18) performed a study of IL-2 toxicity modulation by LSF. IL-2 was administered as a 5-day continuous i.v. infusion at 18 mIU/m^2/day followed by 5 days of rest and 5 additional days of infusion at 6 mIU/m^2/day. The target LSF plasma levels of 9 μM were based on murine data for the inhibition of macrophage activation by lipopolysaccharide and protection against endotoxin lethality (data on file at CTI). The plasma levels achieved ranged from 3.2 to 10.2 μM. In this study, as in the prior study with i.v. PTX, there was an apparen

reduction in nephrotoxicity (lower creatinine peak and duration of creatinine elevation and higher serum HCO_3 nadir level) and a LSF dose-dependent reduction in IL-2-induced weight gain compared with historical controls. The only major toxicity attributed to LSF was a prolonged ventricular arrhythmia in a patient with extensive tumor involvement of the mediastinum, airway, and carotid artery (13). In subsequent studies at higher dose levels, there has been no cardiac arrhythmia attributable to LSF (data on file at CTI).

It is likely that the lack of toxicity modulation by LSF in our study was due to subtherapeutic plasma levels despite the selection of a dose based on the levels achieved in Phase I studies (10–12). Because the pharmacological analyses were not performed until after the completion of our study, the data were not available for individual pharmacologically based dosing or for alteration of the LSF dose for the entire cohort early in the study. Although the results of the C18 (unsaturated):C16 acyl chain ratio analysis suggested that even the plasma LSF levels achieved in our patients were capable of down-modulating the target signaling pathways, there is evidence to suggest that this end point was not achieved and that the consequent lipoperoxide-mediated tissue damage was incompletely suppressed (23). For example, Fig. 3 demonstrates that, although LSF decreases to some extent the augmentation in individual acyl chain concentrations induced by IL-2 (from >600 to 130–150%), it does not succeed in completely abrogating increases in acyl chains. The presence of such persistent increases in linoleate may account for the lack of clinical benefit from LSF.

The ratio of the C18 unsaturated fatty acids linoleate and oleate to the C16 saturated acid palmitate has been used as a measure of cytokine-mediated phospholipid signaling pathways. In a recent study of septic patients at risk for ARDS, patients who progressed to develop ARDS had significantly increased ratios of C18:C16 in comparison with healthy controls. In a subset of these patients randomized between LSF (1.5 mg/kg qid) *versus* no LSF, there was a significantly lower C18:C16 ratio in the septic patients treated with LSF *versus* the non-LSF-treated septic patients. The authors did not state whether progression to develop ARDS was influenced by LSF treatment in this small cohort. However, the individual acyl chain increases in these patients were of lower magnitude than those which we observed in the present study, implying that high-dose IL-2 is a potent inducer of free fatty acids (24).

Other clinical studies attempting to modify the toxicities mediated by IL-2 and other inflammatory cytokines have also been disappointing. In a recent study of patients receiving high-dose IL-2 for the treatment of advanced malignancy similar to those in the present trial, the toxicity-modulating activity of a TNF-neutralizing molecule (TNFR:Fc) was compared with placebo. The treatment end points were similar to those selected for our trial. High serum TNF levels occurred in both patient groups, but the activity of TNF was neutralized in patients receiving TNFR:Fc, as measured in a fibroblast cytotoxicity assay and by a reduction in serum levels of IL-6, IL-8, and IL-1 receptor antagonist. Nevertheless, clinical toxicities were unaffected by treatment with TNFR:Fc (25).

The aggregate data suggest that successful modulation of the multisystem toxicities of cytokine therapy and related syndromes has not been achieved by the current approaches. Furthermore, it is likely that some if not all of the phenomena responsible for the multiorgan toxicities of high-dose cytokine states (generally described by the term "capillary leak syndrome") depend on the same biochemical pathways responsible for the antitumor effects. Therefore, although it is likely that more potent inhibitors or combinations of agents will be identified, rigorous preclinical investigations will be essential to assure that successful modulation of organ toxicities is not accompanied by a loss of antitumor activities. These regimens will then need to be studied in large randomized clinical trials like the present study to confirm that the clinical outcomes can also be improved.

APPENDIX

Statistical Methods. In general, all demographic and analytical between-group differences were analyzed parametrically using the ANOVA and nonparametrically using the Wilcoxon test and the ANOVA of ranks. The Cochran/Mantel-Haenzel χ^2 was used for analyzing contingency tables. The log-rank test was used to analyze right-censored data for the time to occurrence of a selected event or grade of toxicity. The reported *P*s are uncorrected for the multiplicity of tests performed.

Twenty-eight toxicities were evaluated using the BRMP scale. The worst severity recorded, the number of days to the worst severity, and the duration of the worst severity are summarized for each treatment interval. Treatment group differences for the worst severity are assessed using the Cochran/Mantel-Haenzel test. For the number of days at the worst severity, treatment group differences are assessed using the ANOVA of ranks.

The five BRMP toxicities, serum creatinine, hypotension, neurological, cardiac, and pulmonary toxicities (by grade), plus serum creatinine levels, are included in the analysis. The maximum change in serum creatinine from the first dose through the sixth day of CT1501R treatment, as well as the maximum severity for each of the BRMP toxicities over the same time period, is determined for each patient.

In addition, a composite score is calculated for each patient that considers both the severity of these five BRMP toxicities and how long each toxicity was severe or worse over the first 6 days of treatment. This is called the average total score. Each day of CT1501R treatment is assigned a value of 1 on days where the BRMP toxicity had a grade of 2 or less, 3 on days where the grade was 3, and 4 when the grade was 4. The last score is carried out to the sixth day to guarantee that every patient had 6 days of scores. These scores are added up through the first 6 days of CT1501R treatment, totaled over the five BRMP toxicities, and then divided by 6 to give the average total score.

To keep the type I error rate at the 0.05 level of significance, the primary efficacy parameter analysis uses the randomization distribution to determine a *P* for treatment differences. This approach incorporates treatment differences in each of the individual maximum BRMP toxicities, the maximum change in serum creatinine, and the parameter included using a one-sided Wilcoxon statistic. The overall randomization distribution *P* is found as follows: (*a*) determine the minimum of the *P*s for maximum change in serum creatinine and for neurological, hypotension, cardiac, and pulmonary BRMP toxicities; (*b*) calculate a randomization-distribution *P* for this minimum *P*; (*c*) determine the minimum of the *P*s from the average total score and the randomization distribution *P* above; and (*d*) calculate a randomization-distribution *P* for this new minimum *P*. This last randomization *P* is what we report for the primary efficacy parameter analysis.

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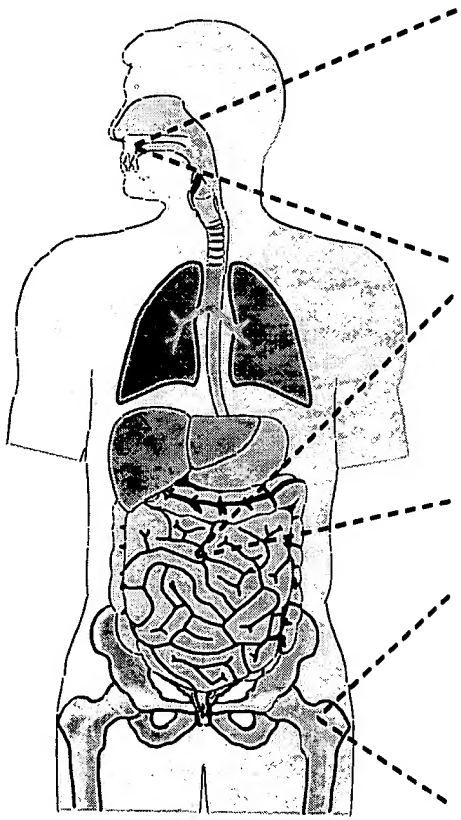
APPENDIX B Side Effects of Cancer Therapy

cti



Cancer Therapy	*Major Side Effect*	*Major Complication*
Oral Lining Cells	Barrier Breakdown (mucositis)	Serious and Fatal Infections
Gut Cells		
Bone Marrow	↓ Infection Fighting WBC's (neutropenia)	

APPENDIX C LSF Phase II Results: BMT

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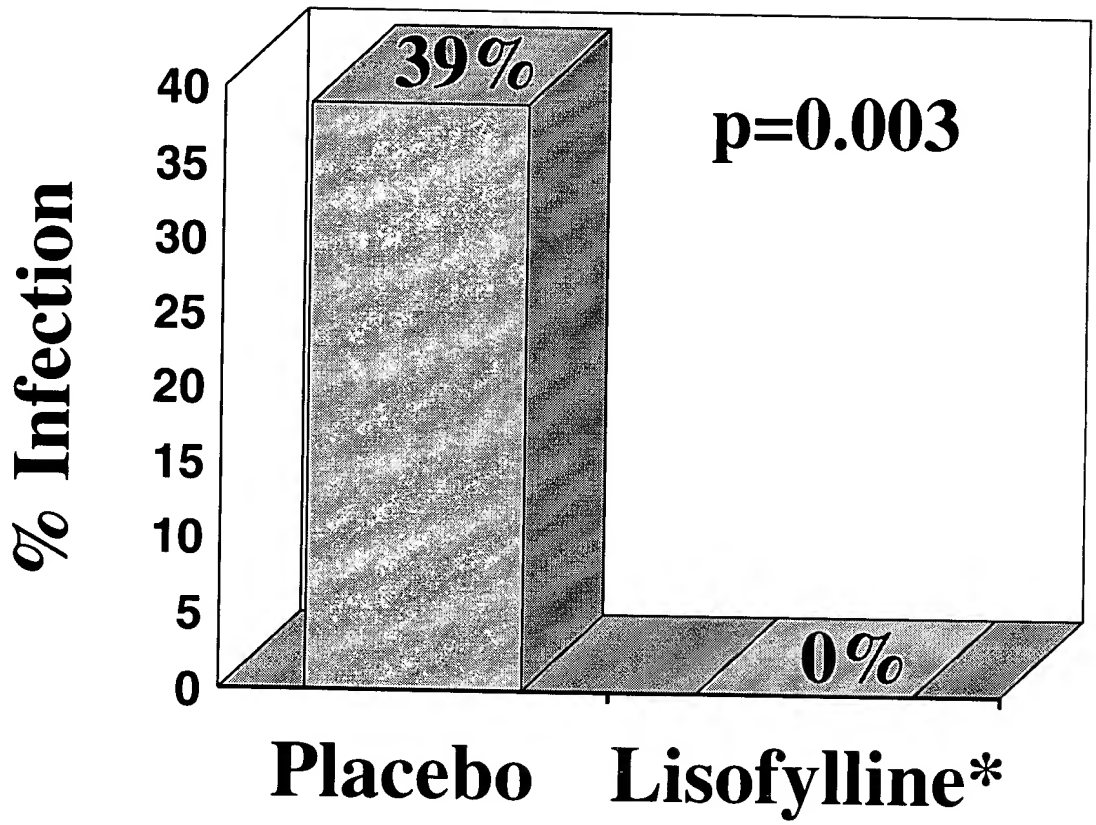
Significantly Decreases All Neutropenic Infections



(*3 mg/kg dose, days 0-35 post BMT)

APPENDIX D LSF Phase II Results: AML

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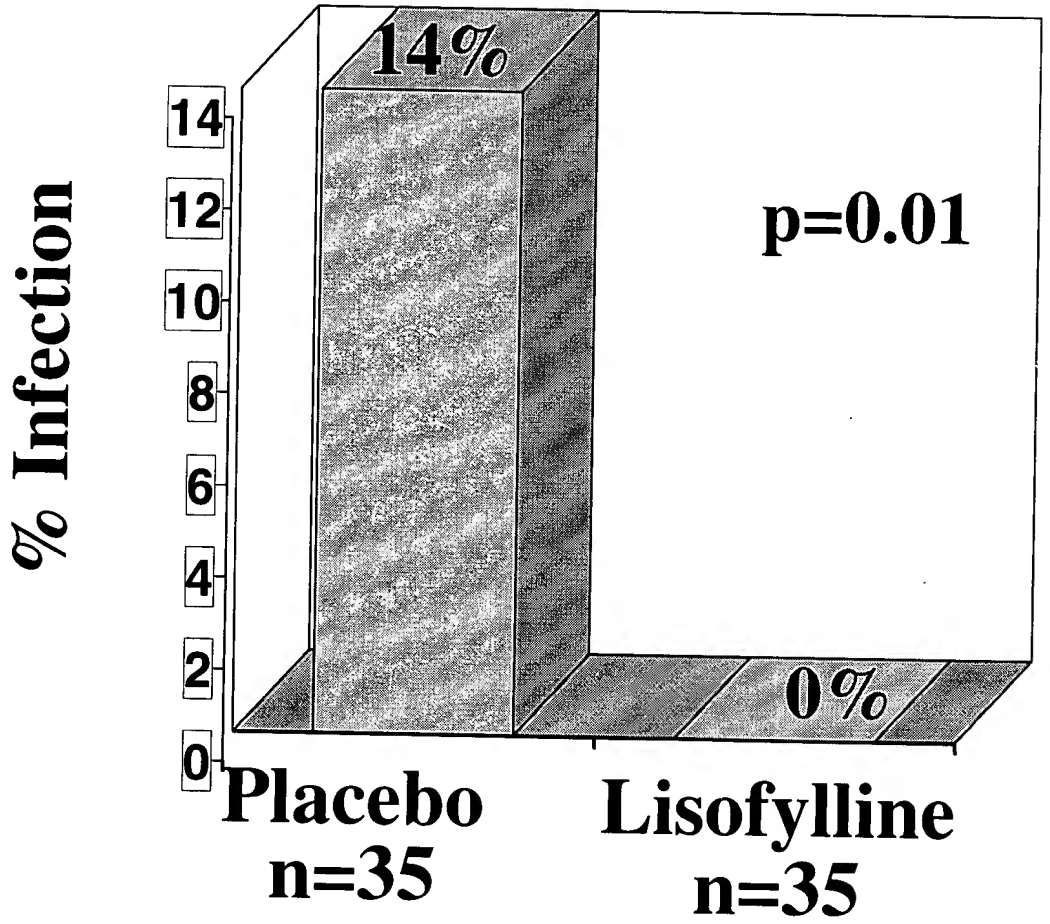
Significantly Decreases Neutropenic Fungal Infections



APPENDIX E

Abstract #2127 - 2:15, Monday, December 7, 1998 - Room C123-124
INFECTION AND IMMUNE RECONSTITUTION

LISOFYLLINE (LSF): A PHASE III DOUBLE-BLIND RANDOMIZED TRIAL FOR THE PREVENTION OF INFECTION AND ACUTE GVHD AFTER MATCHED SIBLING ALLOGENEIC BMT

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LSF, by decreasing the formation of oxidized lipids, has been shown to sustain gut integrity and decrease infection, regimen-related toxicity, acute lung injury, mucositis and acute GVHD in pre-clinical and phase I/II studies. In a phase III study, 132 BMT patients, at 17 treatment centers were randomized to receive LSF, 3 mg/kg IV over 10 minutes every 6 hours (N = 65) or placebo (N = 67) between 8/96 and 10/97. LSF was begun just before the conditioning regimen and continued until 21 days after BMT or until hospital discharge. Stratification parameters were age (< vs. ≥40 years), SWOG performance status (0 vs. 1 or 2), disease status (early vs. advanced) and the use of prophylactic quinolone antibiotics (yes vs. no). Patients received unmanipulated HLA-identical sibling marrow. GVHD prophylaxis was standardized to cyclosporine and methylprednisolone but the cytotoxic therapy was not protocol mandated. Demographics of the treatment groups were imbalanced for significant co-morbidities at the time of BMT (25/65 LSF vs. 12/67 placebo; p = 0.01) and more LSF patients had high-risk disease, were older, and treated with more intensive preparative regimens. The median duration of study drug administration was 28 days in both study arms. Nausea and vomiting were more common in the LSF patients. Overall survival, treatment related mortality and disease relapse were not significantly different by arm. Infections, either serious neutropenic (LSF = 10 vs. placebo = 8, p = 0.58) or serious non-neutropenic (LSF = 16 vs. placebo = 19, p = 0.69) were not significantly different. Grade II-IV acute GVHD was more frequent in the placebo group (LSF = 20 vs. placebo = 31, p = 0.07, Fisher's exact test). The Kaplan-Meier estimate of the probability of grade II-IV acute GVHD was 39% and 51% (p = 0.19, log-rank) respectively for LSF and placebo. An increased risk for GVHD has been associated with older age and more intensive treatment regimens. For patients less than age 40, the probability of acute GVHD was identical for both the LSF and placebo groups (39%). However, for patients of age ≥40, the probability of acute GVHD was 35% and 61% respectively for LSF and placebo (p = 0.025, log-rank). When patients receiving the most intense conditioning regimens (three drugs or two drugs + TBI) were analyzed, acute GVHD occurred in 29% of the LSF patients and 65% of the placebo patients (p = 0.054, log-rank). Further evaluation of LSF is ongoing in matched unrelated donor BMT and further study of LSF in patients undergoing matched sibling BMT is warranted.

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